TITLE OF THE INVENTION

Methods and Devices for Treating Severe Peripheral Bacterial Infections

BACKGROUND OF THE INVENTION

(1) Field of the Invention

The present invention relates to novel methods and devices for treating severe bacterial infections, such as septicemia or bactermeia, using an extracorporeal adsorption container. The device has a solid support disposed and confined within the container and a binding means associated with the solid support that is specific for affixing an infecting bacterium that is causing the severe peripheral bacterial infection and/or affixing bacterial toxins from the bacterium. By passing the infected blood through the container, at least a portion of the infecting bacterium and/or bacterial toxins are removed. The treated blood is returned to the patient.

(2) Description of the Related Art, Including Information Disclosed Under 37 CFR 1.97 & 1.98

Bacterial infections are becoming a greater danger, whether or not caused by accidental or intentional exposure. Certain bacteria have become resistant to antibiotic treatment, in some cases to a number of antibiotics, either naturally or through genetic manipulation. Septicemia is now among the most common causes of death in the United States of America (13th as of the year 2000), accounting for over ten billion dollars annually in health care costs. Fatality rates for septicemia are around 20%, totaling over 50,000 deaths annually.

In some cases a bacterium infects a person in a manner that makes any infection dangerous. Inhalation anthrax (*bacillus anthracis*) infection can be such a case. If inhaled, anthrax spores can cause a set of non-specific symptoms (malaise, fatigue, myalgia, and fever) that do not lead to a clinical diagnosis of anthrax infection, absent actual knowledge of an

anthrax exposure having taken place. The spores are deposited in the alveolar spaces and transported to mediastinial lymph nodes by lymphatic action. Once in the nodes, the spores can transform to vegetative cells. With germination, disease follows rapidly into a severe peripheral bacterial infection.

Replicating bacterium can release toxins that lead to necrosis, edema, and hemorrhage. (For the purposes of the present invention, toxins can also refer to any factors that lead to an actual toxin, such as anthrax edema factor (EF, a 89kD adenylate cyclase protein) that leads to edema toxin (ET) if combined with anthrax protective antigen (PA, a 83kD cell binding component) or anthrax lethal factor (LF, a 90kD metalloprotease) which leads to lethal toxin (LT) if combined with PA.) At this point, often refrred to as the secondary phase, diagnosis typically does not save the patient. In fact, antibiotic treatment may actually cause a crisis in the blood that leads to death, by killing the infecting bacteria, and thereby releasing a flood of toxins to the peripheral system, a toxin overload.

Extracorporeal devices have been used in the past, but not for treating patients for severe peripheral bacterial infections. For example, U. S. 6,039,946 to Strahilevitz discloses an extracorporeal affinity adsorption device for providing therapeutic intervention. The container contains a chelant for binding metal ions in the blood and an antibody specifically binding to either an anti-cancer drug or a combined anti-cancer drug/targeting antibody.

Extracorporeal devices have also been disclosed for use in the treatment of retroviral diseases such as HIV infection. U. S. 4,824,432 teaches about a container that has a means for removing interferon or HIV virus.

BRIEF SUMMARY OF THE INVENTION

The present invention relates to novel methods and devices for treating severe bacterial infections using an extracorporeal adsorption container. The device has a solid support disposed and confined within the container and a binding means associated with the solid support that is

specific for affixing an infecting bacterium that is causing the severe peripheral bacterial infection and/or affixing bacterial toxins from the bacterium. By passing the infected blood through the container, at least a portion of the infecting bacterium and/or bacterial toxins are removed. The treated blood is returned to the patient, whether it is a human or an animal.

In particular, the emergency bacterium and/or toxin removal (EBTR) device for treating a patient having a severe peripheral bacterial infection comprises an extracorporeal adsorption container having an inlet means and an outlet means for circulating blood in a whole or separated form. A solid support is disposed and confined within the container. A binding means is associated with the solid support that is specific for affixing an infecting bacterium that is causing a severe peripheral bacterial infection, thereby allowing for the removal of at least a portion of the infecting bacterium and the return of the treated blood to the patient. For the purposes of the present invention, "severe peripheral bacterial infection" includes the patient having a level of either a bacterium or a mycobacterium in the peripheral system that the use of an antibiotic at that stage of infection puts the patient at a significant risk of induced bacteremia or septicemia from the killing of the infecting bacterial load and/or the peripheral levels of associated bacterial toxins, and also includes the patient having a level of bacterium that is antibiotic resistant, either from environmental exposure or genetic manipulation of the bacterium or mycobacterium. The term also refers to such infections wherein the level of toxins released from the infecting microbe have reached a stage where the patient is at risk from the effects of the toxin on the body, including hemorraghic or edemic destruction of cells. Examples of severe peripheral bacterial infections include an infecting microbe (bacterium or mycobacterium) from the *bacillus*, *meningococcus, streptococcus*, *staphylococcus*, or *paratuberculosis* species. The detection of bacterial infections can be determined by a number of conventional diagnostic means.

A variety of conventional solid supports are suitable for the present invention, including beads, fibers, or membranes. Typically, one should use a support capable of holding a large load of binding means, preferably enough to remove at least one mg of bacteria. The support preferably has a surface area to volume ratio of at least about 4 to 1. For convenience, one can size the container so as to provide enough binding capacity to remove a predetermined amount of

bacteria from the patient, enabling the treating physician to estimate the number of containers necessary to treat an assayed level of infection.

The binding means also can be conventional means for binding to an infecting bacterium and/or associated toxins. Typically, the binding means is adsorbed or bonded to the solid support in an amount sufficient to remove at least 1mg of infecting bacteria or the associated toxins, if present. Suitable binding means include immunoadsorbents such as Con A, lectins, monoclonal antibodies, or polyclonal antibodies. In certain embodiments one can combine at least two different binding means together in one container, such as either binding means for two separate bacterium or for a bacterium and at least one toxin produced by that bacterium. Alternatively, one can provide for a series of containers, each with a separate binding means. In that vein, one can provide for containers that can attach to each other in a serial fashion. For example, each end can be provided with a threaded inlet or outlet port so that a container containing a binding means for a bacterium can be threaded onto a container for the associated bacterial toxins, if desired.

Another variant of the present invention can include the EBTR having an erythrocyte retention means between the inlet and outlet means. The function of this means is to segregate at least the red blood cells from the binding means. For example, one can use a cylindrical tangential flow membrane in the device whereby the blood enters within the interior of the membrane cylinder. The membrane porosity is sized such that erythrocytes stay within that interior space, but the membrane allows the passage of any blood components of a lesser size such as bacterium and bacterial toxins. The binding means is placed outside the membrane. Thus, if any bacterium or bacterial toxins are present in the blood flow, they will migrate through the membrane and can be captured by the binding means. The membrane provides a separation between the red blood cells and the binding means. The shape of these elements should be configured such that at the outlet of the device, filtrate from the membrane and the retentate from the membrane containing the red blood cells can be recombined.

An object of the present invention also is to provide for methods for treating a patient having a severe peripheral bacterial infection. The first step is to connect an extracorporeal

adsorption container as described above to the patient's peripheral system. The patient's blood is circulated through the container, thereby cleansing the blood by removing at least a portion of the infecting bacterium and/or the associated bacterial toxins. The treated blood is returned to the patient. Typically, the blood is treated until the bacterial load has been reduced to a level such that the use of an antibiotic does not put the patient at a significant risk of induced bacteremia or septicemia. To speed up the patient recovery and reduce the risk of bacterial overload, one can pump the blood through the extracorporeal container. Pumping should be done with conventional techniques that preserve erythrocyte integrity.

In some cases, it is preferred to monitor the blood for either the reduction in the level of bacteria or the associated toxins after a set treatment period. Also, any antibiotic treatment of the patient should be curtailed until the infecting bacterial load has been lowered to an acceptable risk level. One should avoid inducing bacterial toxin overload by killing bacterium and thereby releasing a flood of toxins.

BRIEF DESCRIPTION OF THE DRAWINGS

The FIGURE is a sectional view of the extracorporeal container of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

DESCRIPTION OF AN EBTR UNIT

A preferred embodiment of the extracorporeal adsorption container (10) used in the present invention is shown in the FIGURE. A disposable glass or polypropylene column (12) has a conventional inlet fitting (14) at the proximal end and a conventional outlet fitting (16) at the distal end. Medical grade silicon tubing can be connected to each end. The inlet end can have affixed to it a shutoff valve and a first 14 gauge hypodermic needle. The end of the outlet silicone tubing can have connected to it a blood administration set and a shutoff valve and a second 14 gauge needle.

Inside of the column is the bacterium and toxin binding means and the associated solid support. At the inlet and outlet ends are 80 micron nylon nets (18) for retaining the solid support within the container while allowing blood cells to pass through safely. The solid support comprises agarose particles (20), such as CN-Br activated Sepharose 6B available from Amersham Biosciences (Piscataway, New Jersey). Antibacterial antibodies and anti-bacterial toxin antibodies (22) are affixed to the agarose support by conventional means according to instructions from the manufacturer using sterile solutions and glassware that has been previously sterilized. For example, in the case of an EBTR unit for a severe anthrax infection, one can use affinity-purified goat anti *bacillus anthracis* antibodies and goat anti *bacillus anthracis* toxin antibodies available from Scantibodies Laboratory, inc. (Santee, California).

PRODUCTION OF *BACILLUS ANTHRACIS* ANTIBODIES

To create affinity purified anti *bacillus anthracis* polyclonal antibodies, one first uses killed *bacillus anthracis* available from the Centers for Disease Control (Atlanta, Georgia) as the immunogen for injection into the animal (typically a goat). The killed organism is suspended in a solution of 0.85 M sodium chloride to become the aqueous immunogen for injection. The aqueous immunogen for injection is mixed with an equal volume of Freund's complete adjuvant (a mixture of light mineral oil and mannide monooleate and inactivated *mycobacterium tuberculosis bacilli*). The resulting mixture is homogenized to produce an aqueous/oil emulsion for injection into the animal for the primary immunization. The immunogen dose is approximately 100-500 micrograms of bacillus anthracis. The goats are injected monthly with the same dose of immunogen complex except no *mycobacterium tuberculosis bacilli* is used in these subsequent injections. The goats are bled monthly under sterile conditions, starting approximately three months after the primary immunization. The serum (or antiserum) is derived from each bleeding by separating under sterile conditions the red blood cells from the blood by centrifugation and removing the antiserum, rich in antibodies against the *bacillus anthracis*.

To purify the antiserum for the desired antibody against *bacillus anthracis*, one packs a chromatography separation column with heat killed *bacillus anthracis* bound to cross linked agarose beads (such as CN-Br activated Sepharose 4B from Amersham Bioscience, Piscataway, New

Jersey) according to the instructions from the manufacturer using sterile solutions and glassware that has been previously sterilized. The column (which also has been previously sterilized) is packed with the *bacillus anthracis* bound to agarose and the column is washed and equilibrated with sterile 0.01 M phosphate buffered saline (PBS). The antiserum is 0.22 micron filtered and loaded onto the column and washed with sterile 0.01 M PBS in order to remove antibodies that are not against *bacillus anthracis*. The bound specific goat anti *bacillus anthracis* polyclonal antibody is eluted from the solid phase *bacillus anthracis* in the column by passing an elution solution of sterile 0.1 M glycine hydrochloride buffer, pH 2.5 through the column. The eluted polyclonal antibody is neutralized after it leaves the column with either the addition of sterile 1 M phosphate buffer, pH 7.5 or by buffer exchange with sterile 0.01 M PBS under sterile conditions, as is known to those of skill in the art. This affinity-purified goat anti *bacillus anthracis* polyclonal antibody is further 0.22 micron filtered and stored at 2-8 degrees centigrade.

One can repeat the above procedure so as to make affinity-purified goat antibodies against the associated *bacillus anthracis* toxins, namely, protective antigen, edema factor, lethal factor, edema toxin and lethal toxin.

The affinity purified goat anti *bacillus anthracis* antibodies are bound to cross linked agarose beads (CN-Br activated Sepharose 6B which is available from Amersham Bioscience, Piscataway, New Jersey) according to instructions from the manufacturer using sterile solutions and glassware that has been previously sterilized.

PRODUCTION OF AN EBTR UNIT

One can produce an EBTR unit suitable for use with a patient or domesticated animal in the following manner. A 200 ml glass chromatography column with inlet and outlet connectors and 80 micron nets at both inlet and outlet ports is sterilized. Sterile medical grade silicone tubing is attached to both the inlet and outlet of the column. A sterile shutoff valve is attached to the inlet tubing and a blood administration set with shutoff valve is attached to the outlet tubing. Needles (14 gauge) are attached to the ends of the inlet and outlet tubing. One gram of the affinity-purified goat anti *bacillus anthracis* antibody bound to 200 ml of Sepharose 6B agarose beads is packed into the column. A peristaltic pump is attached onto the inlet tubing and the column is washed with sterile

saline. With the sterile saline in place in the inlet and outlet tubing and the column, the shutoff valves are closed and the sterile unit is sealed under sterile conditions. An EBTR unit to remove products of the *bacillus anthracis* (i.e., toxins) is made by filling the column with goat antibodies to *bacillus anthracis* toxins (PA, EF, or LF) are bound to agarose beads (produced in a manner analogous to the goat anti *bacillus anthracis* antibodies described above). Typically, a 200 ml EBTR unit is capable of removing about one gram of an infecting bacterium.

PATIENT SELECTION FOR THE USE OF AN EBTR UNIT

Often a patient having a peripheral bacterial infection is not clinically diagnosed until the infection progresses into a severe peripheral bacterial infection. While it is possible to use an EBTR unit soon after a bacterial infection occurs, practically, in most cases the infection will not be identified until it is severe. For example, a *bacillus anthracis* infected patient typically will have passed into the secondary phase of infection by the time of diagnosis, and as such, is a candidate for the present invention. Candidacy can also be made by employing a rapid quantitative assay of the blood level of for a particular infecting agent, such as *bacillus anthracis* and/or quantitative rapid tests for the toxic byproducts of the infecting agent, for *bacillus anthracis*, namely, protective antigen, edema factor, lethal factor, edema toxin and lethal toxin. These rapid assays have the advantage of providing objective quantitation to the process of selecting patients for treatment. The selection process using the quantitative rapid test for *bacillus anthracis* can be based on a fairly low level of infecting bacteria. The administration of bacteriocidal antibiotics can bring about the accelerated release of life threatening toxins, *i.e.*, the patient can die from toxin loads, even if the bacteria has been substantially reduced or effectively eliminated. The selection process using the quantitative rapid test for the products of *bacillus anthracis* is based on the critical life threatening threshold level of toxins already in the patient's blood. As in the case of establishing LD50 (lethal dose at which 50% of a population would die) one can determine separate threshold cutoff's for differing infections.

TREATING A PATIENT WITH AN EBTR UNIT

Before using the EBTR unit such as described above, a patient is injected with about 100 units per kilogram of patient body weight of sodium heparin, available from Wyeth-Ayerst (Philadelphia, Pennsylvania). To use the EBTR unit, the container is removed from its sterile sealed

packaging and the inlet silicone tubing is connected to a blood peristaltic pump capable of delivering 100-300 ml per minute of blood, available from Baxter Healthcare (Deerfield, Illinois). The patient is placed in a supine position and both of the skin points of entry for the arm brachial veins are wiped with appropriate sterilant. The bottom inlet needle is inserted into one of the brachial veins of the patient and the pump speed is increased to 50 ml/min to allow blood to fill from the bottom the EBTR unit. The EBTR unit is rotated to assure that no air is trapped in the unit. When blood has filled the EBTR unit and the blood administration set and with no air in the outlet line, the 14 gauge outlet line needle is inserted into the patient's other brachial vein. The pump speed is increased up to 300 ml/min. During the EBTR treatment the patient's levels of *bacillus anthracis* and the levels of the products of *bacillus anthracis* are quantitatively assessed by quantitative rapid tests.

During the EBTR treatment, rapid quantitative tests can be used to assay blood levels of *bacillus anthracis* and levels of *bacillus anthracis* toxins. Typically, one would not treat the patient with antibiotics while using the EBTR unit, so as to avoid the release of further toxins into the peripheral system. The use of EBTR treatment can be halted when the infection and toxin levels have reached a point where the use of antibiotics will not set the patient at risk from subsequent release of *bacillus anthracis* toxins. One should note that one does not have to remove substantially all infecting bacterium or bacterial toxins from the patient, though this is preferable. Alternatively, one can discontinue EBTR treatment when the levels are low enough that typically the patient's immune system is able to overcome immunologically residual *bacillus anthracis* levels and clear the associated toxins naturally. Of course, such a decision should be made by the attending physician and is specific for each patient, depending upon numerous known factors. Due to the potential infectious nature of anthrax, the used EBTR unit is incinerated.

The ordinarily skilled artisan can appreciate that the present invention can incorporate any number of the preferred features described above.

All publications or unpublished patent applications mentioned herein are hereby incorporated by reference thereto.

Other embodiments of the present invention are not presented here which are obvious to those of ordinary skill in the art, now or during the term of any patent issuing from this patent specification, and thus, are within the spirit and scope of the present invention.